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DATA EVALUATION RECORD

9/1/1999

Pirimicarb

Study Type: 81-8; Neurotoxicity Screening Battery in Alpk:AP₇SD Rats

Work Assignment No. 1-01-24 (MRID 44485301)

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity Study in Rats
OPPTS Number: 870.6200

OPP Guideline Number: §81-8(a)

DP BARCODE: D243438
P.C. CODE: 106101

SUBMISSION CODE: S537943
TOX. CHEM. NO.: 359C

TEST MATERIAL (PURITY): Pirimicarb (97.6% a.i.)

SYNONYMS: 5,6-Dimethyl-2-dimethylamino-4-dimethylcarbamoyl-oxy-pyrimidine

CITATION: Horner, S.A. (1996). Pirimicarb: Acute Neurotoxicity Study in Rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report ID CTL/P/5232. November 26, 1996. MRID 44485301. Unpublished.

SPONSOR: Zeneca Ag Products, 1800 Concord Pike, Wilmington, Delaware.

EXECUTIVE SUMMARY: In an acute neurotoxicity screening battery study (MRID 44485301), male and female Alpk:AP_{SD} rats (15 animals/sex/group) were given a single dose of pirimicarb (97.6% a.i.) in corn oil by gavage at levels of 0, 10, 40, or 110 mg/kg. Ten animals/sex/group were observed through day 15. Five animals/sex/group were observed and sacrificed on day 1 for cholinesterase determination.

No differences of toxicological concern were observed in body weights, food consumption, absolute or relative brain weights, or histopathology. In the functional observation battery (FOB), males and females groups were comparable to controls during the pretest evaluation, and no alterations of toxicological significance occurred at days 8 and 15.

At 110 mg/kg, two males and two females died or were sacrificed following dose administration on day 1. Necropsy findings included dark-colored liver, distended stomach and/or fluid contents, and mottled lungs. Clinical signs (# animals) observed outside of the FOB were: hunched posture (males - 4, females - 2), miosis (males - 4, females - 2), signs of salivation (males - 1, females - 6), upward curvature of spine (males - 8, females - 5), chromodacryorrhea (males - 1, females - 2), prostration (females - 1), pinched sides (females - 3), and tip toe gait (females - 2). At test day 1, there were decreases ($p < 0.05$ or 0.01) in males vs controls in brain ($\downarrow 23\%$), erythrocyte ($\downarrow 15\%$), and plasma ($\downarrow 51\%$) cholinesterase activities, and in females vs

controls in brain ($\downarrow 20\%$) and plasma ($\downarrow 47\%$) cholinesterase activities. FOB toxicity observed on day 1 was as follows: Clinical observations: animals exhibited irregular breathing, decreased breathing depth, clonic convulsions, gasping and/or prostration (1/8 females vs 0/10 controls), decreased activity (3/8 females vs 0/10 controls), chromodacryorrhea (1/8 males, 2/8 females vs 0/20 controls), hunched posture and miosis (4/8 males, 2/8 females vs 0/20 controls), salivation or signs of salivation (1/8 males, 7/8 females vs 0/20 controls), sides pinched in (3/8 females vs 0/10 controls), stained around mouth (1/8 males vs 0/10 controls), stained around nose (1/8 males vs 0/10 controls), tip toe gait (2/8 females vs 0/10 controls), upward curvature of spine (8/8 males, 5/8 females vs 0/20 controls), and urinary incontinence or signs of urinary incontinence (1/8 males, 2/8 females vs 0/20 controls). Landing foot splay measurements: decreased foot splay in the females vs controls ($\downarrow 21\%$, $p < 0.01$). Tail flick: increased time to tail flick in the females vs controls ($\uparrow 38\%$, $p < 0.05$). Forelimb grip strength: a non-significant decrease in forelimb grip strength in males and females vs controls ($\downarrow 11-12\%$). Hindlimb grip strength: decreased hindlimb grip strength in females vs controls ($\downarrow 19\%$; not statistically significant). Motor activity: females showed inhibition of motor activity, with 4 of 10 intervals decreased ($p < 0.05$) with respect to controls ($\downarrow 50-83\%$), leading to decreased ($p < 0.05$) overall motor activity ($\downarrow 47\%$).

At 40 mg/kg, one female died following dose administration on day 1. Necropsy findings included discoloration of the caecum, colon, duodenum, jejunum, and ileum, thickened omental vessels, distended stomach, and fluid stomach contents. Clinical signs (# animals) observed outside of the FOB in the mid-dose group were: hunched posture (females - 1), miosis (females - 1), upward curvature of spine (females - 2), and chromodacryorrhea (males - 1). In females on day 1, plasma cholinesterase activity was reduced ($\downarrow 29\%$, $p < 0.05$). In the FOB, animals exhibited toxicity on day 1 as follows: Clinical signs - chromodacryorrhea (1/10 males vs 0/10 controls), hunched posture (1/9 females vs 0/10 controls), miosis (1/9 females vs 0/10 controls), upward curvature of spine (2/9 females vs 0/10 controls), stains around the mouth (1/9 females vs 0/10 controls), and signs of urinary incontinence (1/9 females vs 0/10 controls). Hindlimb grip strength: decreases were observed in the females vs controls ($\downarrow 18\%$; not statistically significant). Motor activity: females had a non-significant decrease in overall motor activity ($\downarrow 32\%$).

One 10 mg/kg female was sacrificed on day 1 for humane reasons. The sponsor stated that the clinical signs and macroscopic findings were consistent with a possible misdosing. Upward curvature was observed in 3/9 low-dose females. No treatment-related findings in cholinesterase activity or during FOB or motor activity evaluations were observed at this dose.

The acute neurotoxicity LOAEL is 110 mg/kg based on effects on clinical signs, cholinesterase activity, landing foot splay and tail flick measurements, and motor activity. The acute neurotoxicity NOAEL is 40 mg/kg.

The submitted study is classified as **acceptable** (§81-8) and satisfies the guideline requirements for an acute neurotoxicity screening battery in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Pirimicarb

Description: White solid

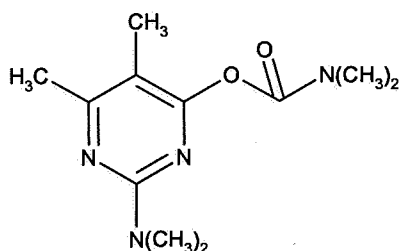
Lot/Batch #: P16

Purity: 97.6% a.i.

Stability of compound: The compound was stable for at least 21 days in corn oil.

CAS #: 23103-98-2

Structure:



2. Vehicle and/or positive control: Corn oil

3. Test animals: Species: Rat

Strain: Alpk:AP₆SD

Age and body weight range at study initiation: 42 days old; 160-212 g (males), 120-160 g (females)

Source: Rodent Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK

Housing: 5/cage

Diet: CT1 diet, Special Diet Services Limited, Stepfield, Witham, Essex, UK, ad libitum

Water: Tap water, ad libitum

Environmental conditions:

Temperature: 21±2°C

Humidity: 55±15%

Air changes: ≥15/hour

Photoperiod: 12 hr dark/12 hr light

Acclimation period: 2 weeks

B. STUDY DESIGN:

1. In life dates - start: 5/21/96 end: 6/7/96

2. Animal assignment: Animals were assigned to treatment groups as indicated in Table 1 using a body weight-dependent randomization process. Ten animals/sex/group were allocated to the main study group. Five animals/sex/group were allocated to the day 1 cholinesterase activity evaluation (satellite group).

Table 1. Study design

Test groups	Dose (mg/kg)	Number of Animals/Sex	
		Main study group	Satellite group
1 (control)	0	10	5
2 (low-dose)	10	10	5
3 (mid-dose)	40	10	5
4 (high-dose)	110	10	5

3. Dose selection: The rationale for dose selection was based on results of a previous study carried out in the same laboratory. No further details were provided.
4. Positive controls - Positive control data were not included in this submission; however, acceptable positive control data for acrylamide (MRID 43013305) had been previously reviewed by the Agency (DP Barcode, D197441). The study showed that acrylamide induced neurotoxic effects in rats when administered in the diet for a period of 29 days. The study was an acceptable positive control study for ICI Central Toxicology Laboratory, Chesire, UK.
5. Test article preparation and analysis: Test article solutions were prepared by mixing the appropriate amount of pirimicarb (adjusted for purity) with corn oil and were stored at room temperature in the dark. Prior to the start of the study, stability of the test substance in corn oil was evaluated for a period of 21 days at room temperature. Homogeneity (top, middle, and bottom) was evaluated in duplicate samples of the low- and high-dose formulations prior to the study start. Once during the study, samples of test substance formulations were analyzed at all dose levels for concentration.

Results: Homogeneity analysis (% of nominal): 93-106%.

Stability analysis (mean % of day 1): 98-102%.

Concentration analysis (mean % of nominal): 98-102%.

The analytical data indicated that the mixing and storage procedures were adequate and that the variance between nominal and actual dosage to the animals was acceptable.

6. Dosage administration: All doses were administered to fasted animals as a single dose on study day 1 in a volume of 1 ml/100g. Dosing was based on the body weight recorded prior to administration. Control animals received the carrier, corn oil, only.
7. Statistics - Food consumption, motor activity, time to tail-flick, landing foot splay, grip strength, brain parameters, cholinesterase activity, and neuropathy target esterase activity were analyzed using an analysis of variance (ANOVA). Body weight and brain weight parameters with respect to initial values were analyzed using an analysis of covariance.

C. METHODS:

1. Observations: Animals were observed in detail for clinical signs once daily.
2. Body weight: Animals were weighed prior to the study start (days -7 and -1), at study day 1 (immediately before and approximately 3 hours after dosing), study day 8, and 15.
3. Food consumption: Food consumption was recorded continuously and reported as a weekly mean (g/rat/day) for each cage.
3. Functional observation battery (FOB): Ten animals/sex/group were removed from their home cage and subjected to the FOB at day -7, 1 (approximately 3 hours after dosing) and on days 8 and 15. Testing was performed by the same technician, who was unaware of the animal group assignment. The following parameters were observed:

Home cage observations

Posture
Gait
Tremors
Convulsions
Abnormal motor movements

Manipulative observations

Reactivity to handling

Response observations

Auditory response

Open field observations

Posture
Gait
Arousal
Alertness
Stereotypic and bizarre behavior
Tremors
Convulsions
Abnormal motor movements
Emaciation
Dehydration
Hypotonia/hypertonia
Altered appearance

Neuromuscular tests

Hindlimb grip strength
Forelimb grip strength
Landing foot splay
Tail-flick test

4. Locomotor activity: Locomotor activity was measured in the same animals and time points as the FOB, using an automated activity recording apparatus. Each observation consisted of 10 scans of 5 minute duration.
5. Cholinesterase and neuropathy target esterase activities measurements: Cholinesterase activity evaluations were conducted on plasma, red blood cells (RBC), and brain regions in 5 animals/sex/group in the control, 10, 40, and 110 mg/kg groups at study days 1 and 15. The brain was excised and weighed. The left half of the brain was used for brain cholinesterase activity determination; the right half was used for neuropathy target esterase activity.
6. Sacrifice and pathology:
 - a. Unscheduled deaths: All animals that died or were sacrificed *in extremis* received a complete necropsy.
 - b. Brain weights and measurements: At study termination, 5 animals/sex/group were anaesthetized with sodium pentobarbitone, killed by perfusion fixation, and submitted for neuropathological examination. The brain was excised, weighed, and the length and width measured.
 - c. Gross pathological examination: All animals killed during the study were subjected

to a gross pathological examination. Fixed animals were subject to a full pathological examination, which included external observation and internal observation of all organs and structures.

- d. Microscopic examination: The following tissues were taken from animals fixed by perfusion, embedded in a carrier, stained, and examined in the control and high-dose groups by light microscopy:

Central Nervous System		
Brain		
Cerebral cortex	Hippocampus	Cerebellum
Pons	Medulla	
Spinal cord		
Lumbar region with dorsal root ganglia and spinal roots	Cervical region with dorsal root ganglia and spinal roots	
Other		
Gasserian ganglion/Trigeminal nerve		
Peripheral Nervous System		
Sciatic nerve	Sural nerve	Tibial nerve
Other		
Gastrocnemius muscle		

II. RESULTS

A. Observations

1. Mortality - Two high-dose males and 3 females (2 high-dose and 1 mid-dose) died or were sacrificed for humane reasons following dose administration on day 1. In addition, one 10 mg/kg female was sacrificed on day 1 for humane reasons. The sponsor stated that clinical signs and macroscopic findings were consistent with a possible misdosing of the low-dose female.
2. Clinical signs - Treatment-related clinical signs were observed in the mid- and high-dose groups. Observations were similar in nature to those observed in the FOB evaluation (Table 2).

In the high-dose group, the signs manifested were as follows: hunched posture (males - 4,

females - 2); miosis (males - 4, females - 2); signs of salivation (males - 1, females - 6); upward curvature of spine (males - 8, females - 5); chromodacryorrhea (males - 1, females - 2); prostration (females - 1); pinched sides (females - 3); and tip toe gait (females - 2).

In the mid-dose group, the signs manifested were as follows: hunched posture (females - 1); miosis (females - 1); upward curvature of the spine (females - 2); and chromodacryorrhea (males - 1).

Table 2. Selected clinical signs (total occurrence/# animals) from day -7 to 15 in rats dosed with pirimicarb.^a

Sign	Males (mg/kg)				Females (mg/kg)			
	0	10	40	110	0	10	40	110
Total animals	15	15	15	13	15	14	14	13
Hunched	0/0	0/0	0/0	5/4	0/0	0/0	1/1	3/2
Miosis	0/0	0/0	0/0	4/4	0/0	0/0	1/1	2/2
Signs of Salivation	0/0	0/0	0/0	1/1	0/0	0/0	0/0	6/6
Upward Curvature of Spine	0/0	0/0	0/0	9/8	0/0	4/3	2/2	6/5
Chromodacryorrhea	0/0	0/0	16/1	1/1	0/0	0/0	0/0	2/2
Prostrated	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/1
Pinched Sides	0/0	0/0	0/0	0/0	0/0	1/1	0/0	3/3
Tip Toe Gait	0/0	0/0	0/0	0/0	0/0	0/0	0/0	3/2

a Data extracted from the study report, Table 4, pages 42 through 47.

B. Body weight and food consumption: No treatment-related differences were observed in body weights. Food consumption was decreased ($p < 0.01$) for the first week in the mid- (↓3%) and high-dose groups (↓5%), but the differences were minor and not considered to be of toxicological concern.

C. Function observation battery:

1. Clinical observations: Ten animals/sex/group were evaluated. All groups were comparable during the pretest evaluation and at test days 8 and 15. At test day 1, administration of pirimicarb caused clinical toxicity in male and female rats in the 40 and 110 mg/kg groups (Table 3). No treatment-related effects were noted in the 10 mg/kg group. Results from

animals that died during dosing were not reported in this section, but are reported in section II.A.2.

At day 1, high-dose animals exhibited the following: irregular breathing, decreased breathing depth, clonic convulsions, gasping and/or prostration (1/8 females vs 0/10 controls); decreased activity (3/8 females vs 0/10 controls); chromodacryorrhea (1/8 males, 2/8 females vs 0/20 controls); increased breathing rate (1/8 females vs 0/10 controls); hunched posture and miosis (4/8 males, 2/8 females vs 0/20 controls); salivation or signs of salivation (1/8 males, 7/8 females vs 0/20 controls); sides pinched in (3/8 females vs 0/10 controls); stains around mouth (1/8 males vs 0/10 controls); stains around nose (1/8 males vs 0/10 controls); tip toe gait (2/8 females vs 0/10 controls); upward curvature of spine (8/8 males, 5/8 females vs 0/20 controls); and urinary incontinence or signs of urinary incontinence (1/8 males, 2/8 females vs 0/20 controls). Mid-dose animals exhibited the following: chromodacryorrhea (1/10 males vs 0/10 controls); hunched posture (1/9 females vs 0/10 controls); miosis (1/9 females vs 0/10 controls); upward curvature of spine (2/9 females vs 0/10 controls); stains around mouth (1/9 females vs 0/10 controls); and signs of urinary incontinence (1/9 females vs 0/10 controls). In the low-dose animals, upward curvature of the spine was seen in 3/9 treated females.

Table 3. Selected clinical observations in rats at day 1 post-dosing with pirimicarb.^a

Observation	Males (mg/kg)				Females (mg/kg)			
	0	10	40	110	0	10	40	110
Decreased activity	0	0	0	0	0	1	0	3
Irregular breathing	0	0	0	0	0	0	0	1
Decreased breathing depth	0	0	0	0	0	1	0	1
Clonic convulsions	0	0	0	0	0	0	0	1
Chromodacryorrhea	0	0	1	1	0	0	0	2
Gasping	0	0	0	0	0	0	0	1
Hunched posture	0	0	0	4	0	0	1	2
Increased breathing rate	0	0	0	0	0	1	0	1
Miosis	0	0	0	4	0	0	1	2
Diarrhea/signs of diarrhea	1	0	0	1	1	0	0	0
Prostrated	0	0	0	0	0	0	0	1
Salivation/signs of salivation	0	0	0	1	0	0	0	7
Sides pinched in	0	0	0	0	0	1	0	3

Stained around mouth	0	0	0	1	0	0	1	0
Stained around nose	0	0	0	1	0	0	0	0
Tip toe gait	0	0	0	0	0	0	0	2
Upward curvature of spine	0	0	0	8	0	3	2	5
Urinary incontinence/signs of urinary incontinence	0	0	0	1	0	1	1	2

a Data extracted from the study report, Table 7, pages 64 through 74; n=10 animals/sex/dose.

2. Landing foot splay measurements: All groups were comparable during the pretest evaluation and at test days 8 and 15. At test day 1, administration of pirimicarb caused decreased foot splay (Table 4) in the high-dose females ($\downarrow 21\%$, $p < 0.01$). There were no significant differences in any of the other groups.

Table 4. Landing foot splay measurement (mm) in rats at day 1 post-dosing with pirimicarb.^a

Day of study	Males (mg/kg)				Females (mg/kg)			
	0	10	40	110	0	10	40	110
Day 1	57.5 (10)	55.5 (10)	52.5 (10)	56.5 (8)	59.0 (10)	56.8 (10)	57.3 (9)	46.5** (8)

a Data extracted from the study report, Table 8, page 97; Number of animals examined is listed parenthetically.

** Significantly different from controls at $p < 0.01$.

3. Tail flick: All groups were comparable during the pretest evaluation and at test days 8 and 15. At test day 1, administration of pirimicarb caused increased time to tail flick (Table 5) in the high-dose females vs controls ($\uparrow 38\%$, $p < 0.05$). There were no significant differences in any of the other groups.

Table 5. Time to tail flick (seconds) in rats at day 1 post-dosing with pirimicarb.^a

Day of study	Males (mg/kg)				Females (mg/kg)			
	0	10	40	110	0	10	40	110
Day 1	11.6 (10)	12.0 (10)	12.5 (10)	13.4 (8)	11.2 (10)	12.4 (10)	10.4 (9)	15.4* (8)

a Data extracted from the study report, Table 9, page 98. Number of animals examined is listed parenthetically.

* Significantly different from controls at $p < 0.05$.

4. **Forelimb grip strength:** All groups were comparable during the pretest evaluation and at test days 8 and 15. At test day 1, administration of pirimicarb caused a non-significant decrease in forelimb grip strength of 4-7% in the low-dose, 6-9% in the mid-dose, and 11-12% in the high-dose animals (Table 6).

Table 6. Forelimb grip strength (g) in rats at day 1 post-dosing with pirimicarb.^a

Day of study	Males (mg/kg)				Females (mg/kg)			
	0	10	40	110	0	10	40	110
Day 1	705 (10)	675 (10)	665 (10)	625 (8)	643 (10)	600 (10)	583 (9)	563 (8)

a Data extracted from the study report, Table 10, page 99. Number of animals examined is listed parenthetically.

5. **Hindlimb grip strength:** All groups were comparable during the pretest evaluation. At test day 1, administration of pirimicarb to the female animals caused a dose-dependent, non-significant decrease in hindlimb grip strength of 7, 18, and 19% in the low-, mid-, and high-dose groups, respectively (Table 7). At day 8, increases ($p < 0.05$) in hindlimb grip strength were observed in mid- ($\uparrow 20\%$) and high-dose ($\uparrow 18\%$) females vs controls. At day 15, high-dose males showed decreased hindlimb grip strength with respect to controls ($\downarrow 17\%$, $p < 0.05$). However, due to the lack of an effect at day 8, this finding is considered spurious and not of toxicological concern.

Table 7. Hindlimb grip strength (g) in rats post-dosing with pirimicarb.^a

Day of study	Males (mg/kg)				Females (mg/kg)			
	0	10	40	110	0	10	40	110
Day 1	293 (10)	300 (10)	335 (10)	331 (8)	313 (10)	290 (10)	258 (9)	253 (8)
Day 8	513 (8)	460 (10)	538 (10)	516 (8)	435 (10)	400 (9)	522* (9)	513* (8)
Day 15	553 (10)	503 (10)	508 (10)	459* (8)	458 (10)	394 (9)	403 (9)	413 (8)

a Data extracted from the study report, Table 10, page 100. Number of animals examined is listed parenthetically.

* Significantly different from controls at $p < 0.05$.

6. Motor activity: All groups were comparable during the pretest evaluation. At test day 1, administration of pirimicarb caused a slight, non-significant decrease in overall (1-50 minutes) motor activity (Table 8) in high- ($\downarrow 12\%$) and mid-dose ($\downarrow 14\%$) males, with significance ($p < 0.05$) being reached only at minutes 6-10 (high-dose - $\downarrow 30\%$, mid-dose - $\downarrow 24\%$). High-dose females showed greater inhibition of motor activity, with 4 of 10 intervals decreased ($p < 0.05$) with respect to controls ($\downarrow 50$ -83%), leading to decreased ($p < 0.05$) overall motor activity ($\downarrow 47\%$). The mid-dose females also had decreased overall motor activity ($\downarrow 32\%$), although not significantly so. At day 8, high-dose groups had non-significantly increased overall motor activity ($\uparrow 5$ -11%) with respect to controls. At day 15, the overall motor activity was non-significantly decreased in high-dose males and females vs controls ($\downarrow 16$ -19%). These findings at days 8 and 15 are inconsistent and not significant, and therefore, not considered to be of toxicological concern.

Table 8. Mean motor activity in rats at day 1 post-dosing with pirimicarb.^a

Interval (minutes)	Males (mg/kg)				Females (mg/kg)			
	0	10	40	110	0	10	40	110
Number tested	10	10	10	8	10	10	9	8
Day 1								
6-10	58.8	55.2	44.8*	41.0*	48.1	42.1	47.8	23.9*
21-25	6.4	5.2	11.9	10.8	38.2	43.9	15.3*	15.4*
26-30	4.9	8.7	5.7	6.6	40.3	34.1	18.2	15.4*
31-35	14.5	26.4	12.7	4.1	34.3	36.6	11.9*	27.8
41-45	11.9	18.5	11.1	17.0	40.1	34.3	25.1	7.0*
Overall	235.1	258.1	202.2	206.4	388.0	408.5	265.2	206.1*
Day 8								
Overall	203.6	201.6	199.1	212.9	372.2	341.8	327.3	412.6
Day 15								
Overall	381.0	314.5	325.5	308.3	446.2	492.4	413.8	373.9

^a Data extracted from the study report, Table 11, pages 103 through 108.

* Significantly different from controls at $p < 0.05$.

D. Cholinesterase activities determinations:

There were no differences of toxicological concern at day 15. At test day 1, there were decreases ($p < 0.05$ or 0.01) in high-dose males in brain ($\downarrow 23\%$), erythrocyte ($\downarrow 15$), and plasma ($\downarrow 51\%$) cholinesterase activities, and in high-dose females vs controls in brain ($\downarrow 20\%$) and plasma ($\downarrow 47\%$) cholinesterase activities (Table 9). In mid-dose females, plasma cholinesterase activity was also reduced ($\downarrow 29\%$, $p < 0.05$) on day 1. No other toxicologically significant decreases occurred.

Table 9. Cholinesterase activities in rats at day 1 and 15 post-dosing with pirimicarb.^a

Tissue	Males (mg/kg)				Females (mg/kg)			
	0	10	40	110	0	10	40	110
Day 1								
Number tested	5	5	5	5	5	5	5	5
Brain	12.62	12.72	13.84	9.71**	12.04	12.26	11.82	9.68*
Erythrocyte	2482	2330	2292	2106**	2198	2316	2170	2012
Plasma	572	520	497	279**	1016	881	721*	539**
Neuropathy target esterase	946	922	864	900	933	915	928	904
Day 15								
Number tested	5	5	5	3	5	4	4	3
Brain	11.46	12.64	12.83	13.04	12.09	12.70	12.11	11.79
Erythrocyte	2238	2138	2042*	2300	2210	2128	2185	2087
Plasma	570	549	555	542	1005	962	1103	1146
Neuropathy target esterase	905	913	971	874	925	870	822	896

a Data extracted from the study report, Tables 12 and 13, pages 109 through 112.

* or ** Significantly different from controls at $p < 0.05$ or $p < 0.01$.

F. Pathology:

1. Non-perfused Animals

- a) Unscheduled necropsies - One low-dose female, one mid-dose female, two high-dose males, and two high-dose females died on day 1 of treatment. Findings in the high-dose animals included dark-colored liver, distended stomach and/or fluid contents, and mottled lungs. In the mid-dose animals, necropsy findings included discoloration of the caecum, colon, duodenum, jejunum, and ileum, thickened omental vessels, distended stomach, and fluid stomach contents. In the low-dose animals, necropsy findings included excess watery fluid in the abdominal cavity, pale-colored liver, mottled lung, red lymph nodes, distended stomach, fluid stomach contents, discolored stomach contents, and speckled thymus.
- b) Brain weight - No treatment-related changes in absolute or relative brain weights were

apparent in any of the test groups.

- c) Histopathology - No treatment-related lesions were observed in any of the treated groups.

III. DISCUSSION

- A. Investigators' Conclusions - The investigators concluded that a single treatment with pirimicarb in rats at dosages of 10, 40, or 110 mg/kg resulted in neurotoxicity on day 1 (immediately after dosing) in the 40 and 110 mg/kg groups. The toxicity in the 110 mg/kg group included: mortality, clinical signs, effects on landing foot splay, time to tail flick, and locomotor activity, and reductions in food consumption during week 1. Also apparent were inhibition of brain, erythrocyte, and plasma cholinesterase activities. The toxicity in the 40 mg/kg group included: a single mortality, clinical signs, effects on locomotor activity, and reduction in food consumption during week 1. At 10 mg/kg, 2 females showed clinical signs on day 1 only. The NOAEL for neurotoxic potential is 40 mg/kg.
- B. Reviewer's Discussion/Conclusions - Male and female Alpk:AP₁SD rats were given a single oral dose of pirimicarb at levels of 0, 10, 40, or 110 mg/kg. Formulation homogeneity, stability, and concentration analyses confirmed that nominal dosages were achieved.

No differences of toxicological concern were observed in body weights, food consumption, absolute or relative brain weights, or histopathology.

One low-dose female, one mid-dose female, two high-dose males, and two high-dose females died or were sacrificed following dose administration on day 1. Findings in the high-dose animals included dark-colored liver, distended stomach and/or fluid contents, and mottled lungs. In the mid-dose animals, necropsy findings included discoloration of the caecum, colon, duodenum, jejunum, and ileum, thickened omental vessels, distended stomach, and fluid stomach contents. In the low-dose animals, necropsy findings included excess watery fluid in the abdominal cavity, pale-colored liver, mottled lung, red lymph nodes, distended stomach, fluid stomach contents, discolored stomach contents, and speckled thymus. The sponsor stated that clinical signs and macroscopic findings were consistent with a possible misdosing of the low-dose female.

Clinical signs (# animals) observed outside of the FOB in the high-dose group were as follows: hunched posture (males - 4, females - 2); miosis (males - 4, females - 2); signs of salivation (males - 1, females - 6); upward curvature of spine (males - 8, females - 5); chromodacryorrhea (males - 1, females - 2); prostration (females - 1); pinched sides (females - 3); and tip toe gait (females - 2). In the mid-dose group, the signs manifested were as follows: hunched posture (females - 1); miosis (females - 1); chromodacryorrhea (males - 1); and upward curvature of the spine (females - 2).

At test day 1, there were significant decreases ($p < 0.5$ or 0.01) in high-dose males vs controls in brain ($\downarrow 23\%$), erythrocyte ($\downarrow 15\%$), and plasma ($\downarrow 51\%$) cholinesterase activities, and in high-

dose females vs controls in brain ($\downarrow 20\%$) and plasma ($\downarrow 47\%$) cholinesterase activities. In mid-dose females, plasma cholinesterase activity was reduced ($\downarrow 29\%$, $p < 0.05$).

Function observation battery: In the FOB, all groups were comparable during the pretest evaluation, and no alterations of toxicological significance occurred at days 8 and 15. The mid-dose and high-dose exhibited toxicity on day 1 as follows:

Clinical observations: At day 1, high-dose animals exhibited the following: irregular breathing, decreased breathing depth, clonic convulsions, gasping and/or prostration (1/8 females vs 0/10 controls); decreased activity (3/8 females vs 0/10 controls); chromodacryorrhea (1/8 males, 2/8 females vs 0/20 controls); increased breathing rate (1/8 females vs 0/10 controls); hunched posture and miosis (4/8 males, 2/8 females vs 0/20 controls); salivation or signs of salivation (1/8 males, 7/8 females vs 0/20 controls); sides pinched in (3/8 females vs 0/10 controls); stains around mouth (1/8 males vs 0/10 controls); stains around nose (1/8 males vs 0/10 controls); tip toe gait (2/8 females vs 0/10 controls); upward curvature of spine (8/8 males, 5/8 females vs 0/20 controls); and urinary incontinence or signs of urinary incontinence (1/8 males, 2/8 females vs 0/20 controls). Mid-dose animals exhibited the following: chromodacryorrhea (1/10 males vs 0/10 controls); hunched posture (1/9 females vs 0/10 controls); miosis (1/9 females vs 0/10 controls); upward curvature of spine (2/9 females vs 0/10 controls); stains around mouth (1/9 females vs 0/10 controls); and signs of urinary incontinence (1/9 females vs 0/10 controls). In the low-dose animals, upward curvature of the spine was seen in 3/9 treated females.

Landing foot splay measurements: Administration of pirimicarb caused decreased foot splay in the high-dose females vs controls ($\downarrow 21\%$, $p < 0.01$).

Tail flick: Administration of pirimicarb caused increased time to tail flick in the high-dose females vs controls ($\uparrow 38\%$, $p < 0.05$).

Forelimb grip strength: At test day 1, administration of pirimicarb caused a non-significant decrease in forelimb grip strength of 4-7% in the low-dose, 6-9% in the mid-dose, and 11-12% in the high-dose animals.

Hindlimb grip strength: At test day 1, administration of pirimicarb to the female animals caused a dose-dependent, non-significant decrease in hindlimb grip strength of 7, 18, and 19% in the low-, mid-, and high-dose groups, respectively. At day 8, increases in hindlimb grip strength were observed in mid- ($\uparrow 20\%$, $p < 0.05$) and high-dose ($\uparrow 18\%$, $p < 0.05$) females vs controls. At day 15, high-dose males showed decreased hindlimb grip strength with respect to controls ($\downarrow 17\%$, $p < 0.05$). However, due to the lack of an effect at day 8, this finding is considered coincidental and not of toxicological concern.

Motor activity: At test day 1, administration of pirimicarb caused a slight, non-significant decrease in overall (1-50 minutes) motor activity in high- ($\downarrow 12\%$) and mid-dose ($\downarrow 14\%$) males, with significance ($p < 0.05$) being reached only at minutes 6-10 (high-dose - $\downarrow 30\%$, mid-dose - $\downarrow 24\%$). High-dose females showed greater inhibition of motor activity, with 4 of 10 intervals decreased ($p < 0.05$) with respect to controls ($\downarrow 50-83\%$), leading to decreased ($p < 0.05$) overall

motor activity (↓47%). The mid-dose females also had non-significantly decreased overall motor activity (↓32%).

The acute neurotoxicity LOAEL is 110 mg/kg based on effects on clinical signs, cholinesterase activity, landing foot splay and tail flick measurements, and motor activity. The acute neurotoxicity NOAEL is 40 mg/kg.

C. Study deficiencies - No study deficiencies were noted.

Pirimicarb

Neurotoxicity screening battery [81-8(a)]

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